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ISOLATION AND KINETIC CHARACTERISTICS OF STYRENE-DECOMPOSING BACTERIA

Two bacterial strains effectively decomposing styrene at the concentrations ranging from 0.37 to 1.8 $g \cdot m^{-3}$, i.e. *Agrobacterium rhizogenes* and *Pseudomonas aeruginosa*, were isolated. This compound was degraded by them at maximum decomposition rates, i.e. 110.5 and 98.5 $g \cdot m^{-3} \cdot h^{-1}$, respectively. Its biodegradation was very strongly inhibited by the reduction of the culture medium pH to approx. 4.6.

1. INTRODUCTION

One of many environmentally nuisant volatile compounds is styrene. Its world production in 2000 can be estimated to be about 30 million tonnes [1], [2]. From the environmental and economic points of view, a competitive method for reducing emissions of such substances into the atmosphere is the use of natural abilities of microorganisms to biodegrade organic substances [3]–[6]. However, microorganisms that play a key role in the central module of cleaning plants and optimum conditions for their life are poorly known. This repeatedly makes fully deliberate designing and controlling the operation of such plants impossible. This is because these microorganisms in particular need for their growth, apart from carbon contained in almost every pollutant, also nitrogen, phosphorus and sulphur necessary for amino acids synthesis. The typical ratio of biogenic elements, i.e. C : N : P, in a biomass decomposed by bacteria should amount to 200 : 10 : 1 [7].

The present study aimed at isolation of styrene-decomposing bacteria from a biofilter bed used for cleaning the styrene-polluted air, their identification and general characteristics of styrene-biodegradation kinetics in liquid culture media, i.e. in base medium, and deriving ones of a purposely chosen composition.

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2. RESEARCH METHODS

Bacteria isolation from a bed being the packing of the pilot biofilter cleaning styrene waste gases was carried out according to the procedures tested earlier [8], [9]. In the present study, the kinetics of styrene biodegradation by two most active and stable bacteria strains was tested on culture media of different reaction and composition.

Identification of selected bacteria strains was made with two methods, i.e. by the analysis of fatty acid methyl esters (Fatty Acid Analysis) and DNA sequencing (16S Sequencing) by Microbial ID (Newark, DE, USA).

The rate of styrene decomposition by bacteria was tested in eight culture media of different properties (variable pH and nitrogen content, special additives) at styrene concentrations in air ranging from approx. 0.37 to 1.8 $g \cdot m^{-3}$. The set of culture media comprised a base mineral medium and its modifications, consisting in changes in the proportion of phosphates ($Na_2HPO_4 \cdot 12\ H_2O$ and KH_2PO_4) without changing a total phosphorus content. The purpose of these treatments was to receive different pH, to change the content of ammonia nitrogen and to supplement a culture medium with additional components. These culture media are listed below, whereas their composition is presented in table 1.

Table 1

Composition of culture media

Component	Medium number							
	1	2	3	4	5	6	7	8
$Na_2HPO_4 \cdot 12H_2O$	3.78 g	3.78 g	3.78 g	3.78 g	3.78 g	3.78 g	5.10 g	0.00
KH_2PO_4	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g	0.00 g	1.94
NH_4Cl	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g	2.00 g	5.00 g	5.00 g
$MgSO_4 \cdot 7H_2O$	0.20 g	0.20 g	0.20 g	0.20 g	0.20 g	0.20 g	0.20 g	0.20 g
Additive	–	ME 1 cm^3	CE 200 cm^3	YE 1.00 g	glucose 1.00 g	–	–	–
Distilled water	to 1000 cm^3							

Explanations: ME (microelement mixture): dist. H_2O – 500 cm^3; H_3BO_3 – 2.5 g; $(NH_4)_2\ MoO_4$ – 2.5 g; KJ – 0.25 cm^3; NaBr – 0.25 g; $ZnSO_4 \cdot 7\ H_2O$ – 0.1 g; $Al_2(SO_4)_3 \cdot 18\ H_2O$ – 0.15 g; CE (compost extract): 500 g of compost poured into 750 cm^3 of H_2O and left for 24 h at room temperature, then filtered through a filter paper (filtrate supplemented with distilled water to 500 cm^3); YE (yeast extract)

The culture media applied are as follows: mineral medium 1 (base medium with neutral reaction, pH = 7), medium 2 (base medium + microelements, pH = 7), medium 3 (base medium + compost extract, pH = 7), medium 4 (base medium + yeast extract, pH = 7), medium 5 (base medium + glucose, pH = 7), medium 6 (reduced nitrogen content, pH = 7.13), medium 7 (slightly alkaline reaction, pH = 7.52), and medium 8 (acidic reaction, pH = 4.59).

Biodegradation of styrene vapours in the mixtures with air was examined using an experimental system, the core of which was composed of a set of reactors operating in parallel and filled with microorganism suspension in liquid medium (figure 1); 250-cm^3 Dreschler's scrubbers were used as reactors. Into each of 8 scrubbers connected in parallel, 150 cm^3 of different culture medium was inserted (see table 1) and inoculated with 2 cm^3 of selected bacterial strain suspension (inoculum of cultured bacteria washed-off from slants using 3 cm^3 of physiological salt solution). A non-inoculated culture medium 1 was inserted into the 9th scrubber (control scrubber). Air flow through each scrubber was kept at approx. 0.01 m^3·h^{-1}, whereas styrene concentration was set at a level within the range given above. To ensure the inflow air sterility, before the scrubbers, within the path of gas stream, 0.2 μm Anotop 25 bacterial filters (Whatman, Maidstone, UK) were installed. Styrene concentration in the gas stream was determined by chromatographic method [10].

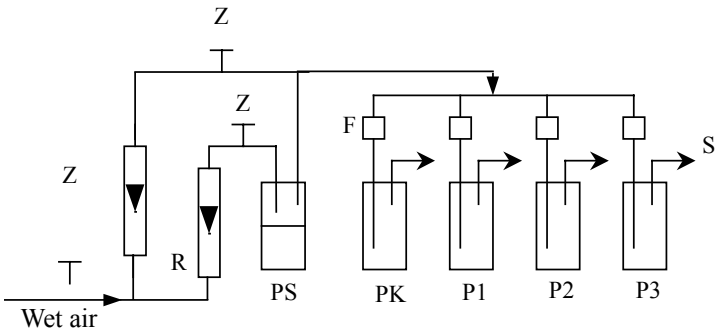

Fig. 1. Schematic drawing of experimental system:
Z – control valves, R – air flowmeters, PS – scrubber with styrene,
PK – control scrubber, F – bacterial filters, P1–P3 – scrubbers with bacterial cultures (max. 9 pcs),
S – scrubber outlets sampling ports

Based on the results of these analyses and the data referring to the flow of styrene vapours and air mixture through the scrubbers, the mass loading of scrubber with styrene and the total efficiency of biodegradation and styrene elimination capacity (specific biodegradation rate) were calculated according to the following equations:

$$M = \frac{G \cdot C_1 \cdot 10^{-3}}{V}, \tag{1}$$

$$S_u = \frac{(C_1 - C_2)}{C_1} \cdot 100, \tag{2}$$

$$EC = \frac{G \cdot (C_1 - C_2) \cdot 10^{-3}}{V}, \tag{3}$$

where:

C_i – the inlet/outlet styrene concentration, $mg \cdot m^{-3}$,
G – the flow rate, $m^3 \cdot s^{-1}$,
V – the suspension volume, m^3,
M – the mass loading of scrubber with styrene, $g \cdot m^{-3} \cdot s^{-1}$,
S_u – the biodegradation efficiency, %,
EC – the elimination capacity (biodegradation rate), $g \cdot m^{-3} \cdot s^{-1}$.

3. RESULTS AND DISCUSSION

During the experiment, 12 bacterial strains capable of decomposing styrene, occurring as the only source of carbon and energy, were isolated. The most active styrene-degrading bacterial strains and, simultaneously, the most stable ones during their storage were *Agrobacterium rhizogenes* and *Pseudomonas aeruginosa.* Taking account of a laboratory practice, in particular of practical applications, storage stability is as much crucial as degradation activity. The results of our measurements of styrene degradation kinetics obtained during experiments in scrubbers are presented in table 2. Linear equations given there represent the dependence of biodegradation rate upon mass loading. As one can see, the bacterial strains cultured in acidic medium, starting from a certain value of substrate loading, did not show any activity at all, while showing only a small activity with lower substrate loadings.

Table 2

Relationships between biodegradation rate (*EC*) and mass loading (*M*)

Culture medium	*Agrobacterium rhizogenes*	*Pseudomonas aeruginosa*
1	$EC = 0.590M - 0.748$	$EC = 0.476M + 12.681$
2	$EC = 0.645M - 7.946$	$EC = 0.476M + 13.997$
3	$EC = 0.112M + 39.610$	$EC = 0.487M + 11.894$
4	$EC = -0.262M + 71.082$	$EC = 0.653M + 5.904$
5	$EC = 0.235M + 30.472$	$EC = 0.447M + 9.869$
6	$EC = -0.084M + 47.107$	$EC = 0.593M + 16.007$
7	$EC = 0.003M + 39.135$	$EC = 0.219M + 31.662$
8	$EC = -0.266M + 37.819$	$EC = -0.024M + 6.717$

Mean values of the rate of biodegradation in the culture media applied within the range of mass loadings used are presented in figure 2. Based on the analysis of the full set of data, it was possible to draw the remaining conclusions presented below.

In the case of *Agrobacterium rhizogenes*, the rate of styrene biodegradation was the highest in the culture medium supplemented with a compost extract (medium 3). Maximum biodegradation rate in this medium reached 110.5 $g \cdot m^{-3} \cdot h^{-1}$ at styrene in-

flow concentration of 1851.1 mg·m^{-3}. As stated by ARNOLD et al. [11], natural beds like peat or compost have the advantage over many others, since they are rich in organic matter, which enables the development and growth of microorganisms.

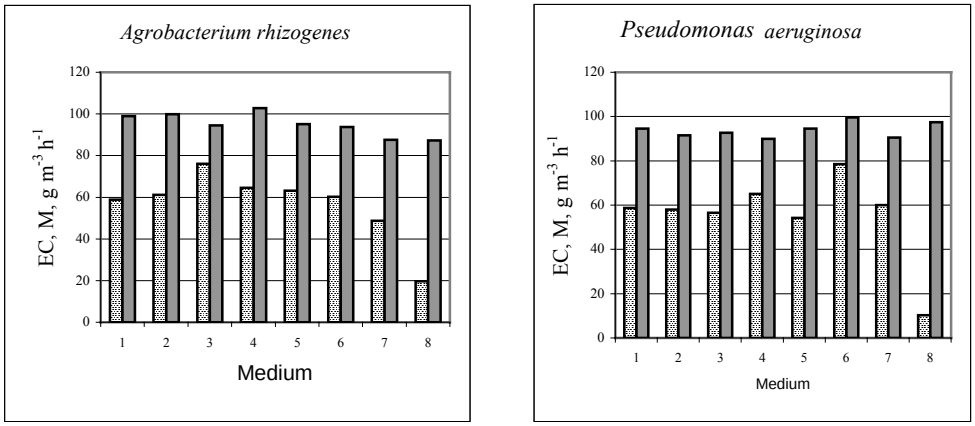

Fig. 2. Mean elimination capacity (*EC* – ⋮) and mean mass loading (M – ▮) during styrene biodegradation in different culture media with *Agrobacterium rhizogenes* (styrene concentration of approx. 1302 mg·m^{-3}) (a) and *Pseudomonas aeruginosa* (styrene concentration of approx. 985 mg·m^{-3}) (b)

On the other hand, the maximum rate of styrene degradation by *Pseudomonas aeruginosa* was observed in the culture medium with reduced nitrogen content (medium 6). This maximum rate, i.e. 98.5 g·m^{-3}·h^{-1}, was recorded for two different styrene concentrations at the reactors inlet, namely 958.4 and 1742.5 mg· m^{-3}. Also the efficiency of styrene degradation by both *Agrobacterium rhizogenes* and *Pseudomonas aeruginosa* was the highest in this culture medium. In the case of both *Pseudomonas aeruginosa* and *Agrobacterium rhizogenes*, it decreased together with an increase in styrene concentration.

The study of biofiltration by ARNOLD et al. [11] showed that microorganism activity was affected mostly by nitrogen and phosphorus. On the other hand, CÁRDENAS-GONZÁLEZ et al. [12] pointed to the fact that a given chemical element is not always available for microorganisms. The nutrient availability depends on its form in a culture medium. Readily-soluble nitrogen and phosphorus compounds, such as NH_4NO_3 and KH_2PO_4, are more easily assimilated by microorganisms and strongly affect the increase of their activity, but only for a relatively short period of time. This was probably a results of their washing out from the biofilter bed. The introduction of the easily available nitrogen forms into culture medium, or even better its complex fertilization, may also restore the degradation capacity of bed-inhabiting microorganisms that would appear to be irretrievably lost [13]–[15].

4. CONCLUSIONS

1. Two bacteria strains: *Agrobacterium rhizogenes* and *Pseudomonas aeruginosa* effectively degraded styrene and showed high storage survivability.

2. Environmental conditions and the content of basic nutrients in the culture medium significantly affected the kinetics of styrene decomposition. The pH reduction always led to a decrease in styrene degradation rate.

3. The maximum rates of styrene biodegradation by *Agrobacterium rhizogenes* and *Pseudomonas aeruginosa* were 110.5 and 98.5 $g \cdot m^{-3} \cdot h^{-1}$, respectively.

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WYIZOLOWANIE I CHARAKTERYSTYKA KINETYCZNA BAKTERII ROZKŁADAJĄCYCH STYREN

W czasie badań wyizolowano dwa szczepy bakterii, to jest *Agrobacterium rhizogenes* i *Pseudomonas aeruginosa*, rozkładających efektywnie styren w zakresie stężeń od 0,37 do 1,8 $g \cdot m^{-3}$. Związek ten był degradowany z maksymalnymi szybkościami 110,5 and 98,5 $g \cdot m^{-3} \cdot h^{-1}$. Szybkość biodegradacji znacząco malała po obniżeniu pH pożywki do około 4,6.